GELATIN BASED EMULSION HYDROGELS AS A MATRIX FOR CONTROLLED DELIVERY

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

Bachelor of Technology (Biotechnology Engineering)

Submitted
By

SARADA PRASANNA MALLICK
Roll No. 107BT018

Under the Guidance of
Dr. Kunal Pal

Department of Biotechnology & Medical Engineering
National Institute of Technology
Rourkela 769008

DEPARTMENT OF BIOTECHNOLOGY & MEDICAL ENGINEERING, NATIONAL INSTITUTE OF TECHNOLOGY-ROURKELA

Dated: May 15, 2011

CERTIFICATE

This is to certify that the thesis entitled "**GELATIN BASED EMULSION HYDROGELS AS A MATRIX FOR CONTROLLED DELIVERY**" submitted by **Mr. SARADA PRASANNA MALLICK** in partial fulfillment for the requirements for the award of Bachelor of Technology Degree in Biotechnology at National Institute of Technology, Rourkela is an authentic work carried out by him under the supervision of the undersigned.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University / Institute for the award of any Degree or Diploma.

(Dr. KUNAL PAL)
Assistant Professor

ACKNOWLEDGEMENTS

I owe a great many thanks to great many people who helped and supported me for the completion of this project effectively and moreover in time.

My deepest and sincere thanks to **Dr. Kunal Pal,** Assistant Professor, Department of Biotechnology & Medical Engineering, National Institute of Technology, Rourkela for giving me an opportunity to carry out this project under his supervision. He has been very kind and patient to me while suggesting the outlines of the project and has also been very helpful in the successful completion of the same. I thank him for his overall support.

I am equally thankful to the Ph.D scholars, Mr. Sateesh Sagiri and Ms. Beauty Behera, Department of Biotech & Medical Engineering, National Institute of Technology Rourkela for their support and guidance.

Finally, let me say "Thank You" to my friends Mr. Ankurman Shrestha and Mr. Prasanjeet Pattnaik for their encouraging words and motivation.

Lastly I express my abysmal adoration and heartfelt devotion to my beloved parents for their countless blessings, unmatchable love, affection and incessant inspiration that has given me strength to fight all odds and has shaped my life and career till today.

In the end I must record my special appreciation to my almighty who has always been source of my strength, inspiration and my achievements.

Date: ***Sarada Prasanna Mallick***

CONTENTS

List of Figures

List of Tables page no

ABBREVIATIONS

%	Percentage
h	Hour
L	Liter
GS	Gelatin Solution
SO	Sunflower Oil
EHs	Emulsion Hydrogels
GA	Glutraldehyde reagent

Abstract

The study describes the development of gelatin based emulsion hydrogels with different proportions of sunflower oil. During the preparation of emulsion hydrogels, the microstructures of the emulsions were studied under light microscope. The hydrogels were characterized by light microscopy, swelling property, mucoadhesivity, pH, D.C impedance measurements and hemocompatibility studies. Salicylic acid was incorporated within the oil phase and its release behavior was studied. The microscopy of the emulsions indicated that the droplet size distribution becomes mono-dispersed as the proportion of the oil is increased. The microscopy of the emulsion hydrogels showed distorted oil droplets for all the samples. The swelling property, mucoadhesivity, dc impedance measurements and release rate of salicylic acid was found to dependent on the oil proportion. All the samples were found to have pH in the range of 6.00-7.00 and were hemocompatible in nature indicating its probable use as controlled delivery vehicles.

Chapter 1

INTRODUCTION AND OBJECTIVE

1.1.Introduction

Emulsions are defined as biphasic and heterogeneous systems containing two immiscible liquids [1-2]. One of the liquids forms the internal phase and is dispersed in the continuous liquid phase [3]. Depending on the polarity of the liquid phases, the emulsions may be categorized either as water-in-oil or oil-in-water emulsions [4]. Usually, they are thermodynamically unstable systems [5-6]. The emulsions may be made thermodynamically stable by using surfactants which help in reducing the interfacial tension amongst the two immiscible phases [7-9]. But the stability of the emulsions depends on the performance of the surfactant molecules. In recent years, some authors have tried to improve the stability of the emulsions by immobilizing the internal phase by increasing the viscosity of the external phase using various polymer matrices. If the final texture of such an emulsion is semi-solid in nature, the emulsions are regarded as emulsions gels (EGs) [10-12]. If the continuous phase is polar in nature then the product is regarded as emulsion hydrogels (EHs) otherwise it is regarded as emulsion organogels [13-14]. In 1980s, EGs were used in food industry as texture modifiers [15-17]. Of late, they have been used to develop drug delivery vehicles. EHs have been extensively investigated to ascertain its properties to deliver hydrophobic drugs [18]. Various hydrophobic drugs (e.g. probucol, S-312-d (a potent Ca^{2+} blocker), itraconazole, griseofulvin) have been successfully incorporated within the EHs [19-21].

Gelatin, a biopolymer, is obtained from animal sources (e.g. bovine, porcine or fish collagen) [22]. It has been extensively used in devising various products of biomedical importance due to its non-toxic, non-carcinogenic, non-immunogenic and biodegradable nature [23]. It dissolves in water at temperatures >35 ^{o}C and forms a gel at temperatures ~30 ^{o}C, depending on the method of production of gelatin [24]. Since the gelatin gels have a gel-sol transition at ~30-35 ^{o}C, the cross-linking of gelatin gels have been tried to improve the stability of the products during handling [25-26].

1.1. Objective of the report

In the present study, attempts were made to develop gelatin based EHs by altering the proportions of the gelatin sol and oil. The EHs were characterized thoroughly to determine its suitability as delivery vehicles.

Chapter 2

MATERIALS & METHODS

2.1. Materials

Gelatin and Tween 80 (polyxyethylene sorbitan monooleate) were procured from Himedia, Mumbai, India. Ethanol was obtained from Honyon International Inc., Hong Yang Chemical Corpn., China. Glutaraldehyde (25%, for synthesis; GA) and hydrocholric acid (35%pure) was obtained from Merck Specialities Private Limited Mumbai, India. Salicylic acid (SA) and sodium citrate were procured from Loba Chemie, Mumbai, India. Sunflower oil (SO) was obtained from local market. Goat intenstine and blood were obtained from local Butcher shop. Double distilled water was used throughout the study.

2.2. Preparation of EHs

Twenty grams of gelatin was dissolved in 100 ml of water, whose temperature was maintained at 50 ^{o}C and kept on stirring at 400 rpm, so as to obtain a clear homogeneous sol. Subsequently, 4 ml of tween 80 was added to the sol and the volume of the mixture was made up to 200 ml by the addition of warm water. This resulted in the formation of a 10 % (w/v) gelatin sol (GS).

EHs were prepared by hot homogenizing (at 50 ^{o}C) GS and SO in various proportions (GS:SO ratio being 2:18, 4:16, 6:14, 8:12, 10:10, 12:8, 14:6: 16:4 and 18:2) at 800 rpm for 15 min. To this homogenized emulsion, 1.1 ml of GA reagent (0.5 ml GA+ 0.5 ml ethanol + 0.1 ml HCl) was added and was further stirred for 30 sec. The mixture was then immediately poured into petri-dishes and was allowed to form gels. The EHs, so obtained, were washed thoroughly to wash off any unreacted GA and hydrochloric acid.

The SA-loaded EHs were prepared using 1% (w/v) solution of SA in SO. Rest of the procedure was same.

2.3. Microscopic evaluation of the hot emulsions and gels

The microstructures of the hot emulsions were studied by under compound light microscope (CH20i, Olympus, India). The microscopic images of the emulsions were analyzed using ImageJ and NI Vision Assistant softwares. After the conversion of hot emulsions to EHs, their microstructures were also analyzed.

2.4. Swelling Behavior

EHs were cut into 1 cm x 1 cm pieces and were weighted accurately (W_o). The pieces of the EHs were dipped into a beaker containing 100 ml of water. At regular intervals of time, 15 min for the first 1h and thereafter 30 min up to 8h, the EHs were taken out from the water, wiped and weighed accurately (W_t). Swelling ratio (SR) of the EHs was calculated as per the formula given in equation 1.

$$SR = \frac{(W_t - W_0)}{W_0} \quad (1)$$

Where SR=swelling ratio

W_t is the weight of the swollen gel at time.

W_0 is the initial gel weight

2.5. Mucoadhesive property

The mucoadhesive property of the samples was analyzed by modified USP disintegration method [27-29]. In short, intestine of freshly sacrificed goats were procured from the local butcher shop. The intestine was immediately transferred to cold saline solution and used within 1 h of collection. The intestinal lumen was cleaned thoroughly by passing saline solution 3-4 times through it. Thereafter, the lumen was cut open and attached to glass slides, such that the intestinal mucosa exposes outwards, using acrylate adhesives. EHs were cut into pieces of 1 cm x 1 cm and were put on the exposed surface of the mucosa. A weight of 5 g was applied over the EHs for 5 min. Thereafter, the vertically slides were put into USP disintegration baskets. Phosphate buffer (pH=7.2) was used as the disintegration medium. The experiment was carried out for 24 h.

Figure 1: Tablet Disintigration apparatus

2.6. pH Measurement

The pH of the EHs were measured using a digital pH meter (model 132E , EI products ,India). The pH probe was kept in touch with the EH samples and the readings were recorded.

2.7. Impedance Measurement

DC impedance of the EHs were measured using a dc impedance meter, which was developed in-house [30]. The impedance meter used a constant current source of 8 µA for the analysis.

2.8. Hemocompatibility test

The hemocompatibility tests were done as per the ASTM standard protocol, which figures out the extent of hemolysis in the presence of the samples [31-40]. For this purpose, fresh goat's blood was collected in the presence of trisodium citrate (TSC) in the proportion of 3.8 g of TSC per 100ml to prevent the coagulation. The citrated blood was diluted with normal saline in the

ratio of 8:10. 0.5 ml of the diluted blood is taken in a centrifuge tube. EH samples of size 5 mm x 5 mm is put into the tube followed by addition of 9.5 ml of saline. For positive control, 0.5 ml of diluted blood is mixed with 0.01 N HCl and subsequently diluted to 10 ml as HCl is having the ability to rupture the R.B.C. For negative control, 0.5 ml of blood is diluted to 10 ml with saline solution. The centrifuge tubes are incubated at 37 ^{o}C for 60 min. the O.D of all the solutions were masured at 545nm using a UV-Visible spectrophotometer. The % hemolysis is calculated as per the following formula:

$$\% \, Hemolysis = \frac{OD_{test} - OD_{Negative}}{OD_{positive} - OD_{Negative}} \times 100$$

If the % hemolysis is <5 then the material is considered as highly hemocompatible, a value <10 indicates hemocompatible whereas a value > 20 indicates non-hemocompatible.

2.9. *In vitro* drug release

SA-loaded samples were used for the study. The drug dissolution tests of the EHs were carried out in single basket dissolution apparatus for 5 h. EHs were cut into 5 cm x 5 cm pieces and were weighed accurately. The drug containing EHs were put into the dissolution basket, containing 900 ml of water. The speed of the over-head stirrer was kept at 100 ± 5 rpm. The temperature of the dissolution medium was maintained at 37 ± 2^{o}C. 3 ml samples were withdrawn at regular intervals of time, 15min for the first hour and 30 min for the remaining time, and were replaced with 3 ml of fresh water. The samples were analyzed at 294 nm using UV-vis spectrophotometer (Double Beam Spectrophotometer, model# 2303, Systronics).

Figure 2: Dissolution Rate Test Apparatus

Chapter 3

RESULTS AND DISCUSSION

3.1. Preparation of EHs

The various compositions of the EHs have been tabulated in table 1. G1 and G2 compositions failed to form gels and were not used for further analysis. The EHs had fruity and alcoholic. The color of the EHs varied from white for G3, where the proportion of the oil was maximum, to various shades of brownish-white depending on the oil proportion. Higher the proportion of the oil was, the whitish tinge of the samples increased. All the samples were opaque.

Table 1: Composition of EHs

Sl. No.	Vol. of gelatin sol (ml)	Vol. of SO (ml)	Odour	Gellation property
G1	2	18	-	No gel formed
G2	4	16	-	No gel formed
G3	6	14	Fruity smell	Gel formed
G4	8	12	Fruity smell	Gel formed
G5	10	10	Alcoholic	Gel formed
G6	12	8	Alcoholic	Gel formed
G7	14	6	Alcoholic	Gel formed
G8	16	4	Alcoholic	Gel formed
G9	18	2	Alcoholic	Gel formed

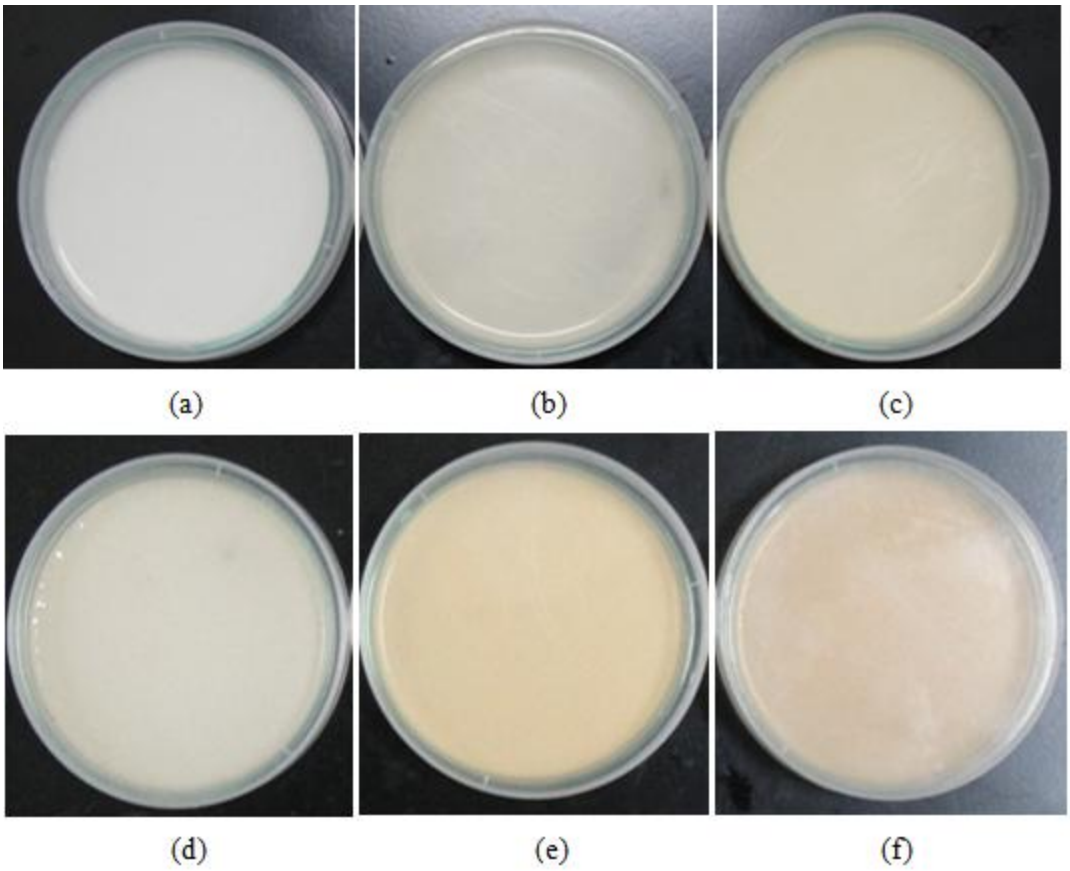
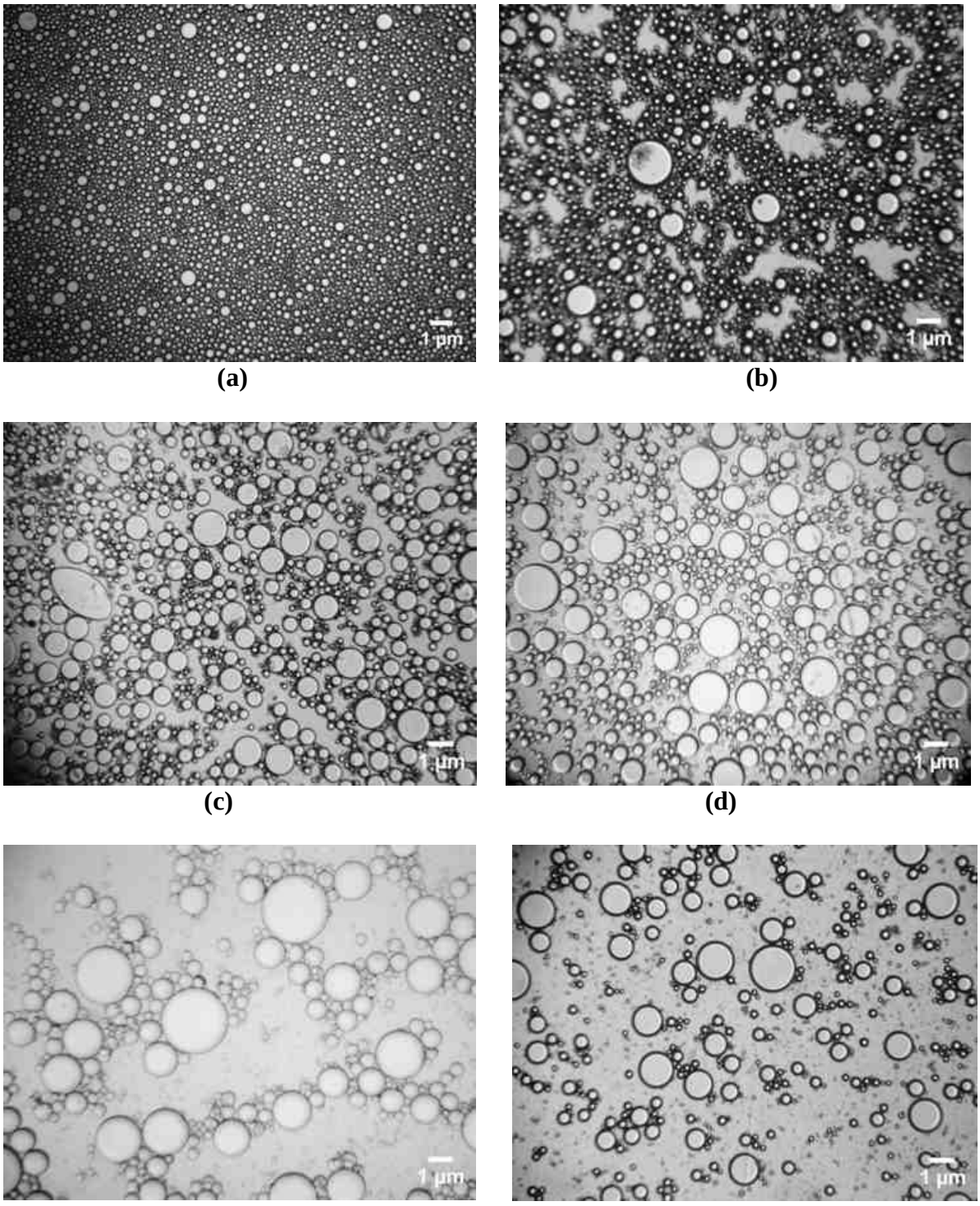


Figure 3: Emulsion hydrogel of different composition of GS and SO (a) G3 (b) G4 (c) G5 (d) G7 (e) G8 (f) G9

3.2. Microscopic evaluation of the emulsion and EHs

The micrographs of the emulsions and EHs have been shown in figures 4 and 5 respectively. The emulsions indicated the presence of dispersed circular SO droplets within the continuous GS. The droplet size distribution of the oil particles indicated that as the proportion of oil was increased, droplet size distribution of the oil was narrow size. Figure 6 indicates the increased size of oil droplets as the oil proportion was decreased. Also, the particle size distribution range became broad, i.e. increased size distribution, as the proportion of oil decreased in the emulsions (figure 4). Once the emulsions were crosslinked, the oil droplets within the crosslinked structures lost their circularity (figure 5).



(a) **(b)**

(c) **(d)**

(e) **(f)**

Figure 4: Light micrographs of SO-in-gelatin sol emulsion. (a) G3, (b) G4, (c) G5, (d) G7, (e) G8 and (f) G9.

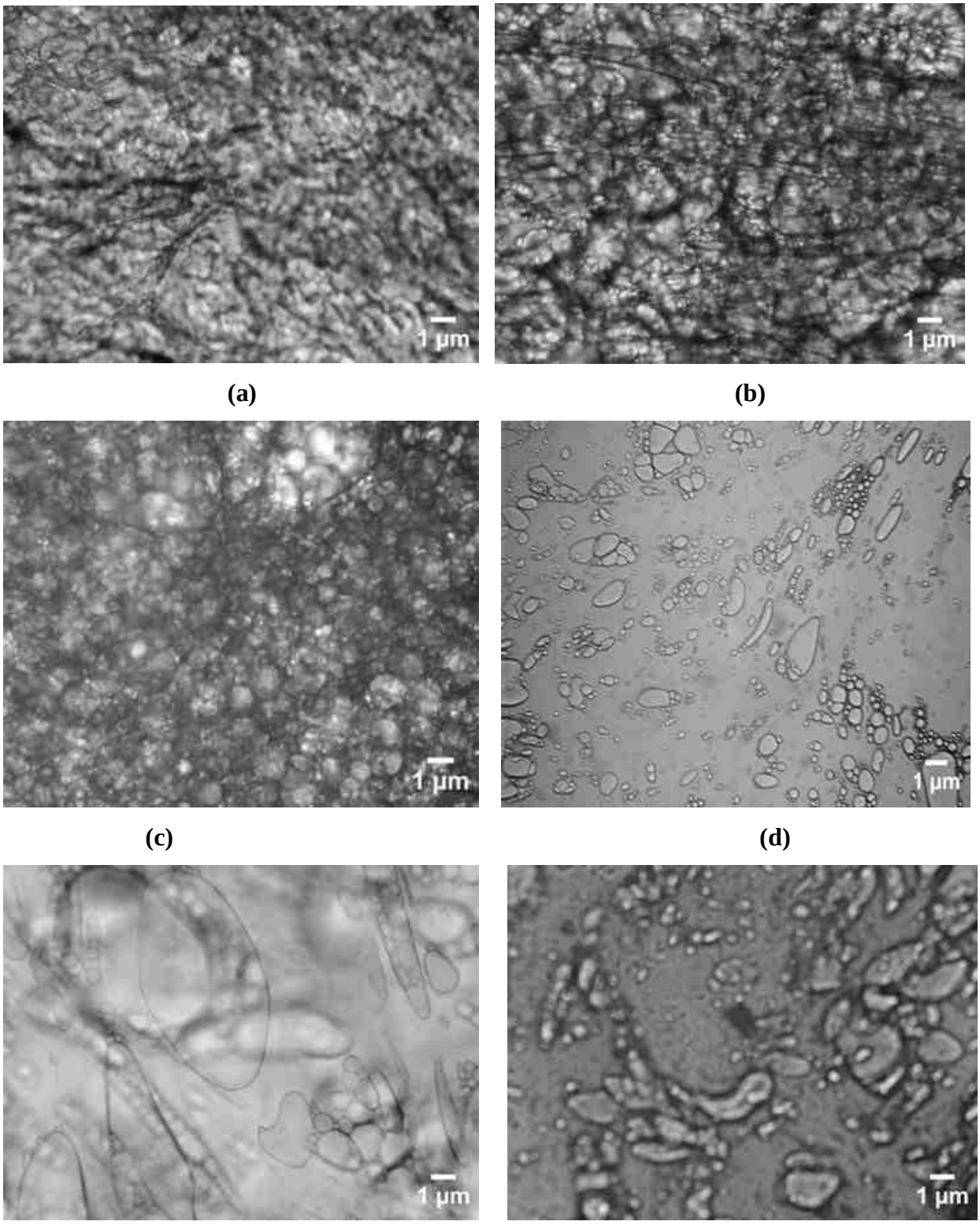


Figure 5: Light micrographs of emulsion hydrogels. (a) G3, (b) G4, (c) G5, (d) G7, (e) G8 and (f) G9.

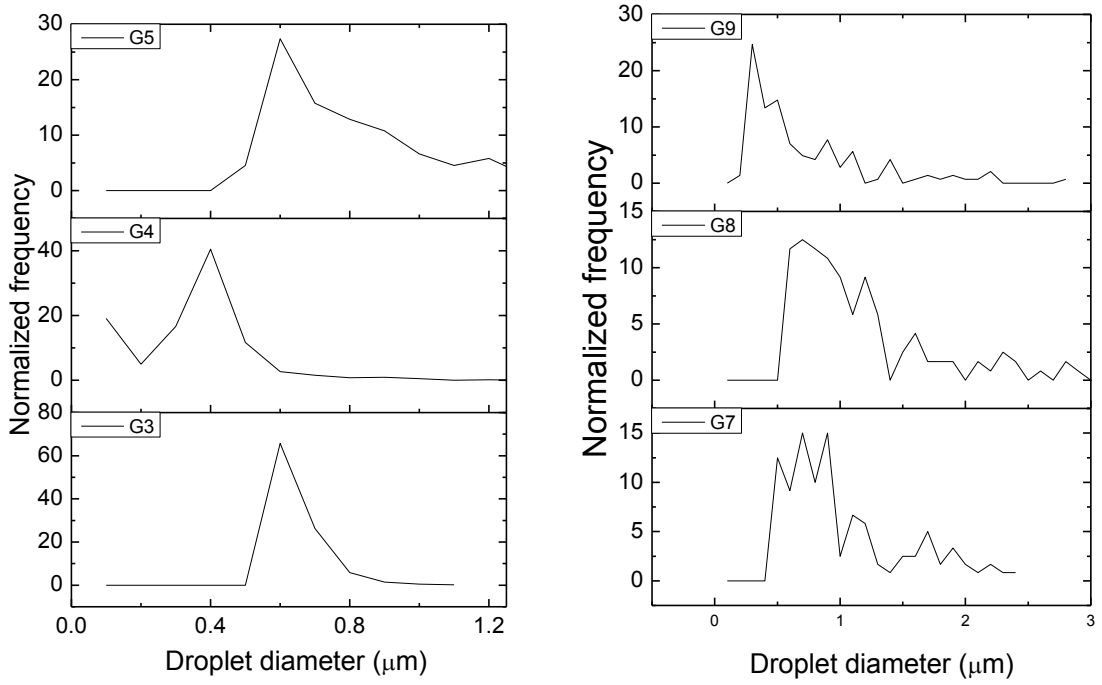


Figure 6: Normalized droplet distribution pattern of SO droplets in emulsions.

3.3. Swelling Behaviour

The swelling behaviour of EHs is shown in figure 7. Swellings of the EHs were dependent on their oil proportions. As the proportion of oil decreased, there was a significant increase in the swelling ratio. This may be attributed to the higher proportions of gel fraction of gelatin present in the samples. It may be estimated from the swelling studies that the rate of release of the drugs dissolved in the oil phase may be higher for the samples with higher water content. Water content in the gels increases for the samples with high swelling ratio due to the increased amount of gelatin. This may be accounted to the partition coefficient effect, which states that the solute distributes itself amongst the two immiscible liquids in a definite concentration ratio [41-43]. The gels were able to maintain their structural integrity even after 8 h of study, though there was some alteration in their texture. This might facilitate the usage of the EHs as implantable delivery devices [44-45].

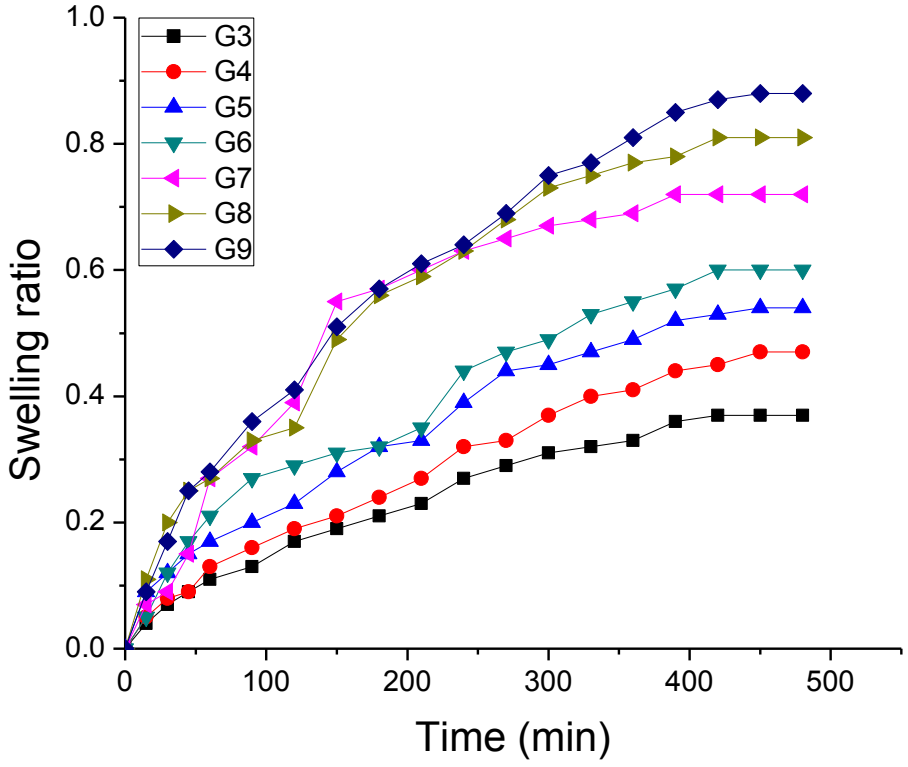


Figure 7: Swelling behavior study of EHs

3.4 Mucoadhesive Property

Mucoadhesive properties of the EHs were analyzed by modified USP disintegration test method, which is basically an *in vitro* wash-off method. The time required to detach the EH samples from the mucosal surface was determined for 24 h. The results showed that G3, G4 and G5 samples got detached at 240 ± 20 min, 360 ± 25 min and 1260 ± 40 min, respectively. The other samples did not detach during the experimental duration. The mucoadhesion is dependent on the crosslinking density of the mucoadhesive polymer. As the proportion of oil increased, there was a subsequent decrease in the gelatin fraction. This lead to the more consumption of the –OH and – NH_2 groups, responsible for mucoadhesion, of gelatin during the crosslinking reaction and hence the lower mucoadhesive property [46].

3.5. pH Measurement

The pH of the biomedical products is important as these are supposed to be in contact with the human tissues. Any variation from the physiological pH may lead to the irritation of the cells, which in turn might cause immunological reactions. For this reason, US pharmacopoeia has set pH standards for transdermal and topical formulations [47]. The results of the EH samples have been tabulated in table 2. The pH of the EH samples was found to be in between 6.00-7.00, indicating their probable use in the formulation of the transdermal or topical products.

Table 2: The pH of EHs

Type of gels	pH±SD
G3	6.57±0.21
G4	6.88±0.30
G5	6.31±0.15
G6	6.43±0.10
G7	6.49±0.20
G8	6.90±0.25
G9	6.80±0.11

3.6. Impedance Measurement

The study indicated that the EHs were able to conduct dc electricity through the samples. The conductance was dependent on the oil proportions. It was found that the impedance of the EH samples increased linearly, with the increase in the oil fraction (figure 8). The conductance of a sample is dependent on the flow of ionic charges through the samples. Increase in the impedance indicates that the flow of these ions is hindered. It may be predicted that as the impedance of the gels are increasing with the increase in the oil fraction, it might result in the decrease in the drug release rate.

Also, gelatin gels have been used as a matrix for iontophoretic delivery systems. Iontophoretic systems employ the use of electric current to improve the drug permeation in the physiological system. It might be possible to alter the release properties of the drugs by altering the composition of the EH samples.

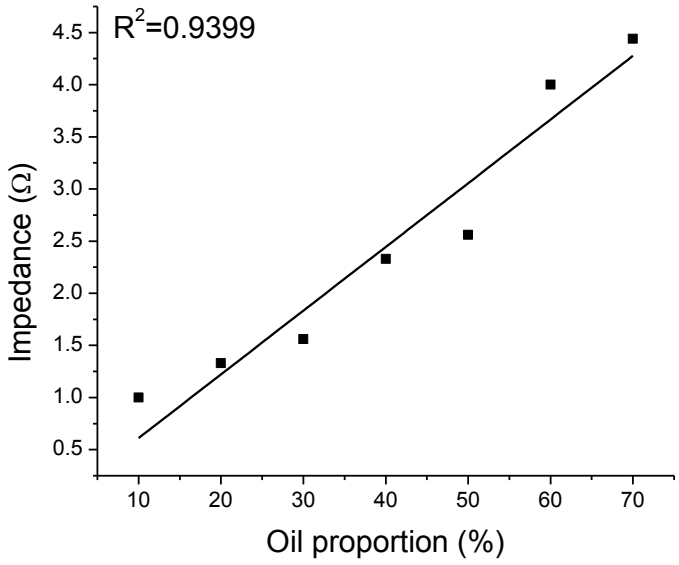


Figure 8: Graph showing the impedance of EHs

3.7. Hemocompatibility

The hemocompatibility of the samples have been tabulated in table 3. The results indicate that the samples are highly haemocompatible in nature indicating its biocompatibility. Hence the EH samples may be tried as a drug delivery vehicle.

Table 3 Hemocompatibility test for EHs

Sample no	$OD_{positive}$	$OD_{negative}$	OD_{test}	% Hemolysis
G3	0.706	0.074	0.078	0.63%
G5	0.706	0.074	0.086	1.9%
G9	0.706	0.074	0.098	3.8%

3.8. *In vitro* drug release

The release profile of SA has been shown in figure 9. The rate of release of the drug was found to be dependent on the composition of the EH samples. From the swelling studies and the impedance measurement studies, it was predicted that the rate of release of the drug will be lower for the samples with higher oil fraction and higher for the samples with lower oil fraction. The release studies confirmed the predictions of the swelling and impedance studies. The % release of SA varied between 80% to 40% in 5h with highest for G9 and lowest to G3, i.e. the rate of release was found to be higher for the samples with lower oil fraction and vice-versa. This can be accounted to higher diffusion rates of water into the networked structure of the samples with lower oil fractions and subsequent relatively quick diffusion of the drugs out of the matrix. Also, higher impedance of the gels with higher oil fraction hinders the diffusion of the solutes through the gel matrix.

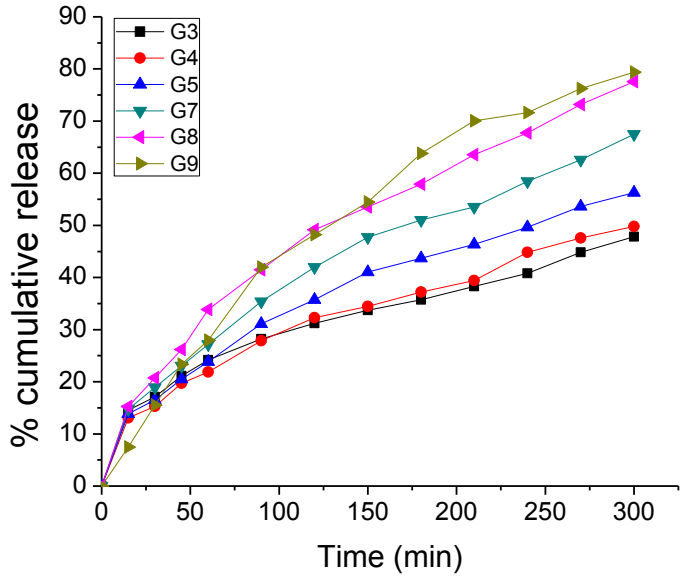


Figure 9. *In vitro* % cumulative release profile of SA from EHs

The release kinetics of SA from the EHs were studied by analyzing for zero order, first order and Higuchian models. The result of kinetics studies have been tabulated in table 4. The results indicated that the release of SA from EHs followed Higuchian kinetics for G3, G4, G6, G7 and G8 samples whereas G9 sample followed first order kinetics. This suggests that the release of the drug from the EHs is diffusion controlled and the EHs may be used as a matrix for controlled delivery.

Table 4: Modelling of release kinetics

Sl. No.	r^2 for model fitting			Best fit
	Zero order model	First order model	Higuchi model	
G3	0.8317	0.8816	0.9823	Higuchi
G4	0.8916	0.9317	0.9945	Higuchi
G5	0.9281	0.9655	0.9951	Higuchi
G7	0.9391	0.9783	0.9945	Higuchi
G8	0.928	0.9773	0.9944	Higuchi
G9	0.9741	0.9951	0.965	First order

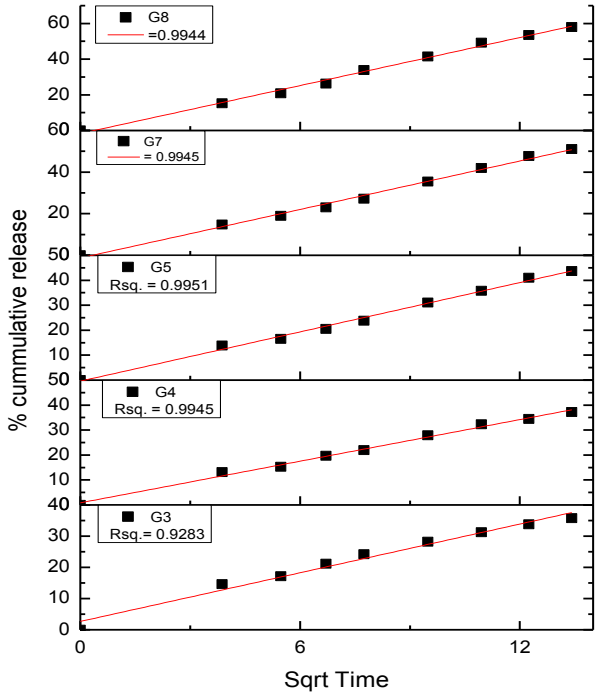


Figure 10: Graphical plot indicating the Higuchian kinetics being followed by G3, G4, G6, G7 and G8 samples.

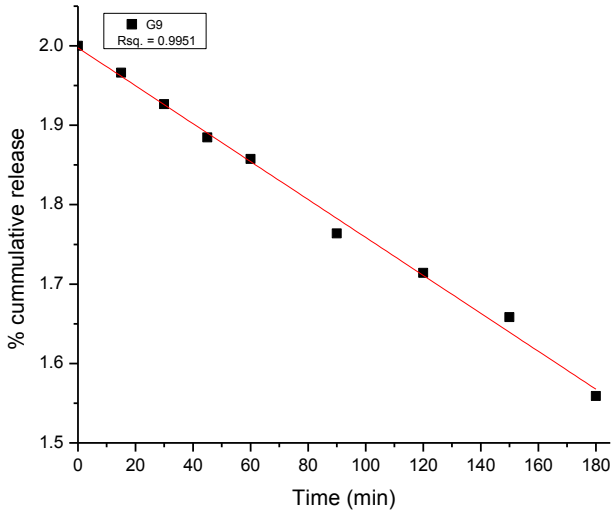


Figure 11: Graphical plot indicating the first-order release kinetics being followed by G9 sample.

Chapter 4

CONCLUSION

4.1. CONCLUSION

The study reports the successful development of gelatin-based EHs. The study indicated that not only the rate of release of the drugs may be modulated but also the mucoadhesive and conductance properties of the gels can be altered by altering the composition of the EHs. The release pattern was highly dependent on the proportion of the aqueous and hydrophobic phases. The gels were found to be hemocompatible and could be tried as a matrix for controlled drug delivery systems.

Chapter 5

REFERENCES

REFERENCES

1. Shinoda, K. and H. Saito, *The stability of O/W type emulsions as functions of temperature and the HLB of emulsifiers: the emulsification by PIT-method.* Journal of Colloid and Interface Science, 1969. **30**(2): p. 258-263.
2. Montagne, F., et al., *Preparation and characterization of narrow sized (o/w) magnetic emulsion.* Journal of magnetism and magnetic materials, 2002. **250**: p. 302-312.
3. Levy, M. and S. Benita, *Drug release from submicronized o/w emulsion: a new in vitro kinetic evaluation model.* International journal of pharmaceutics, 1990. **66**(1-3): p. 29-37.
4. Prasad, R.R.S., *One-step process for transforming a water-in-oil emulsion into an oil-in-water emulsion*. 1986, US Patent 4,627,458.
5. Maini, B., *Foamy Oil Flow In Heavy Oil Prodution.* Journal of Canadian Petroleum Technology, 1996. **35**(6).
6. Julian McClements, D. and S.R. Dungan, *Light scattering study of solubilization of emulsion droplets by non-ionic surfactant solutions.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 1995. **104**(2-3): p. 127-135.
7. Pouton, C.W., *Formulation of self-emulsifying drug delivery systems.* Advanced drug delivery reviews, 1997. **25**(1): p. 47-58.
8. Mathur, A.M., et al., *Polymeric emulsifiers based on reversible formation of hydrophobic units.* Nature, 1998. **392**(6674): p. 367-370.
9. Pearce, K.N. and J.E. Kinsella, *Emulsifying properties of proteins: evaluation of a turbidimetric technique.* Journal of Agricultural and Food Chemistry, 1978. **26**(3): p. 716-723.
10. Dickinson, E. and Y. Yamamoto, *Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase.* Journal of Agricultural and Food Chemistry, 1996. **44**(6): p. 1371-1377.
11. Ravey, J. and M. Stébé, *Structure of inverse micelles and emulsion-gels with fluorinated nonionic surfactants. A small-angle neutron scattering study.* Surfactants and Macromolecules: Self-Assembly at Interfaces and in Bulk, 1990: p. 218-228.
12. Dickinson, E. and Y. Yamamoto, *Viscoelastic Properties of Heat Set Whey Protein Stabilized Emulsion Gels with Added Lecithin.* Journal of food science, 1996. **61**(4): p. 811-816.
13. Tokuyama, H. and Y. Kato, *Preparation of poly (N-isopropylacrylamide) emulsion gels and their drug release behaviors.* Colloids and Surfaces B: Biointerfaces, 2008. **67**(1): p. 92-98.
14. Duffy, N., et al., *Organogel-based emulsion systems, micro-structural features and impact on in vitro digestion.* Journal of the American Oil Chemists' Society, 2009. **86**(8): p. 733-741.
15. Matsumura, Y., et al., *Filler effects of oil droplets on the viscoelastic properties of emulsion gels.* Food hydrocolloids, 1993. **7**(3): p. 227-240.
16. Kim, K., S. Gohtani, and Y. Yamano, *Effect of oil droplets on brittleness of o/w emulsion gel.* Journal of dispersion science and technology, 1997. **18**(2): p. 199-210.
17. Nitta, Y. and K. Nishinari, *Gelation and gel properties of polysaccharides gellan gum and tamarind xyloglucan.* Journal of Biological Macromolecules, 2005. **5**(3): p. 47-52.
18. Sriamornsak, P., N. Thirawong, and S. Puttipipatkhachorn, *Emulsion gel beads of calcium pectinate capable of floating on the gastric fluid: effect of some additives,*

hardening agent or coating on release behavior of metronidazole. European journal of pharmaceutical sciences, 2005. **24**(4): p. 363-373.
19. Schott, H. and A.E. Royce, *Emulsion-based gel and process for preparing same*. 1987, US Patent 4,690,775.
20. Park, K., G. Acharya, and H. Park, *Sol-Gel phase-reversible hydrogel templates and uses thereof*. 2008, US Patent App. 20,090/136,583.
21. Jizomoto, H., et al., *Gelatin-acacia microcapsules for trapping micro oil droplets containing lipophilic drugs and ready disintegration in the gastrointestinal tract.* Pharmaceutical research, 1993. **10**(8): p. 1115-1122.
22. Olsen, D., et al., *Recombinant collagen and gelatin for drug delivery.* Advanced drug delivery reviews, 2003. **55**(12): p. 1547-1567.
23. Narayani, R. and K.P. Rao, *Controlled release of anticancer drug methotrexate from biodegradable gelatin microspheres.* Journal of microencapsulation, 1994. **11**(1): p. 69-77.
24. Quellet, C., H.F. Eicke, and W. Sager, *Formation of microemulsion-based gelatin gels.* The Journal of Physical Chemistry, 1991. **95**(14): p. 5642-5655.
25. Bigi, A., et al., *Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking.* Biomaterials, 2001. **22**(8): p. 763-768.
26. Bigi, A., et al., *Stabilization of gelatin films by crosslinking with genipin.* Biomaterials, 2002. **23**(24): p. 4827-4832.
27. Yadav, V.K., et al., *Effect of magnesium stearate on mucoadhesive microspheres of repaglinide.* International Journal of Pharmaceutical Sciences & Research, 2010. **1**(12): p. 111-118.
28. Bigucci, F., et al., *Pectin based microspheres for colon specific delivery of vancomycin.* Journal of Pharmacy and Pharmacology, 2009. **61**(1): p. 41-46.
29. Dhakar, R.C., et al., *Rosiglitazone maleate microspheres for extending drug release: Formulation and evaluation* International Journal of Pharmaceutical Research and Development, 2010. **2**(10): p. 56-65.
30. Kim, D.H. and D.C. Martin, *Sustained release of dexamethasone from hydrophilic matrices using PLGA nanoparticles for neural drug delivery.* Biomaterials, 2006. **27**(15): p. 3031-3037.
31. Pal, K., S. Bag, and S. Pal, *Development of porous ultra high molecular weight polyethylene scaffolds for the fabrication of orbital implant.* Journal of Porous Materials, 2008. **15**(1): p. 53-59.
32. Pal, K., S. Bag, and S. Pal, *Development and Coating of Porous Ultra High Molecular Weight Polyethylene Plates.* Trends in Biomaterials and Artificial Organs, 2010. **19**(1).
33. Ng, K.W., et al., *Evaluation of ultra-thin poly (-caprolactone) films for tissue-engineered skin.* Tissue engineering, 2001. **7**(4): p. 441-455.
34. Pal, K., A.K. Banthia, and D.K. Majumdar, *Preparation and characterization of polyvinyl alcohol-gelatin hydrogel membranes for biomedical applications.* AAPS PharmSciTech, 2007. **8**(1): p. 142-146.
35. Pal, K., A. Banthia, and D. Majumdar, *Biomedical evaluation of polyvinyl alcohol–gelatin esterified hydrogel for wound dressing.* Journal of Materials Science: Materials in Medicine, 2007. **18**(9): p. 1889-1894.
36. Pal, K. and S. Pal, *Development of porous hydroxyapatite scaffolds.* Materials and manufacturing processes, 2006. **21**(3): p. 325-328.

37. Pal, K., A. Banthia, and D. Majumdar, *Development of carboxymethyl cellulose acrylate for various biomedical applications.* Biomedical Materials, 2006. **1**: p. 85.
38. Sutar, P.B., et al., *Development of pH sensitive polyacrylamide grafted pectin hydrogel for controlled drug delivery system.* Journal of Materials Science: Materials in Medicine, 2008. **19**(6): p. 2247-2253.
39. Pal, K., *Pal K, Banthia AK, Majumdar DK. Preparation and Characterization of Polyvinyl Alcohol–Gelatin Hydrogel Membranes for Biomedical Applications. AAPS PharmSciTech. 2007; 8 (1): Article 21. DOI: 10.1208/pt080121*.
40. Roy, S., et al., *Synthesis of Novel Hydroxypropyl Methyl Cellulose Acrylate—A Novel Superdisintegrating Agent for Pharmaceutical Applications.* Materials and manufacturing processes, 2010. **25**(12): p. 1477-1481.
41. Chiou, C.T., et al., *Partition coefficient and bioaccumulation of selected organic chemicals.* Environmental Science & Technology, 1977. **11**(5): p. 475-478.
42. Barry, B., *Novel mechanisms and devices to enable successful transdermal drug delivery.* European journal of pharmaceutical sciences, 2001. **14**(2): p. 101-114.
43. Shah, N., et al., *Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs.* International journal of pharmaceutics, 1994. **106**(1): p. 15-23.
44. Peppas, N.A., *Hydrogels and drug delivery.* Current opinion in colloid & interface science, 1997. **2**(5): p. 531-537.
45. Li, J., X. Ni, and K.W. Leong, *Injectable drug delivery systems based on supramolecular hydrogels formed by poly (ethylene oxide) s and cyclodextrin.* Journal of Biomedical Materials Research Part A, 2003. **65**(2): p. 196-202.
46. Roy, S., et al., *Polymers in Mucoadhesive Drug-Delivery Systems: A Brief Note.* Designed Monomers &# 38; Polymers, 2009. **12**(6): p. 483-495.
47. Bhatia, V.N. and R.H. Barber, *The effect of pH variations of ointment bases on the local anesthetic activity of incorporated ethyl aminobenzoate. I. Hydrophilic ointment U. S. P.* Journal of the American Pharmaceutical Association, 1955. **44**(6): p. 342-343.